UNCLASSIFIED

AD NUMBER

AD843975

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Critical Technology; 04 JAN 1968. Other requests shall be referred to Commanding Officer, Depart of the Army, Fort Detrick, Attn: Technical Release Branch/TID, Frederick, MD 21701.

AUTHORITY

SMUFD, D/A ltr, 15 Feb 1972

THIS PAGE IS UNCLASSIFIED

AD843975

TRANSLATION NO. 2097

DATE: 4 January 1968

DDC AVAILABILITY NOTICE

Reproduction of this publication in whole or in part is prohibited. However, DDC is authorized to reproduce the publication for United States Government purposes.

STATEMENT #2 UNCLASSIFIED
This document is subject to special export controls and each transmittal to foreign governments or foreign nationals may be made only with prior approval of Dept. of Army, Fort Detrick, ATTN: Technical Release Branch/ TID, Frederick, Maryland 21701

DDC
RECEIVED
NOV 27 1968
C

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

6

RESEARCH ON THE ACTION OF LYSOZYME ON FOWL POX VIRUS

Acta Med. Vet.
(Veterinary Medicine Proceedings)
Vol 11, Jan-Apr 1965, pp 135-140

Persechino Angelo

The action of lysozyme on pox virus was studied by various authors with divergent findings, according to whether the evolution of the disease was in the laboratory or from natural infection. Orfei (reference 1) conducted experiments on the effect of lysozyme on cowpox virus in chicken embryo eggs. In these tests, he found no difference between the lesions of the chorion allantoideum membranes of the eggs inoculated with the virus and lysozyme, and the control group of eggs. These experimental results contrast with those found in clinical practice by Mussa (reference 2) and by Naranjo and De la Torre (reference 3). The former observed a rapid improvement and disappearance of pustules in a 15-month-old baby with widespread infection of cowpox virus. Naranjo and De la Torre observed exanthematic improvement in two patients afflicted with smallpox, after they were treated with lysozyme alone.

With regard to the effect of lysozyme on diphthero-fowl pox virus, the very few references in medical literature are also contradictory. As a matter of fact, while Di Carlo (reference 4) reported that he obtained highly satisfactory results in administering orally lysozyme to a group of canaries suffering from fowl pox, Compagnucci and his colleagues (reference 5), on the contrary, were unable to ascertain the inhibitory action of lysozyme on fowl pox virus of pigeons, either in the case of experiments on egg embryos or in pigeons infected in the laboratory.

The infrequent and contradictory reports and the great lack of experimental data on fowl pox virus ~~have~~ encouraged ~~us to conduct~~ research on chicken egg embryos in an effort to contribute to the knowledge of the effect of the Fleming enzyme on fowl pox virus.

Materials and Methodology

In our experiments we used three-month-old white Leghorn chickens weighing 1 Kg on the average, Wyandotte chicken egg embryos in the eleventh day of incubation, and lysozyme chloride from egg albumin. The solvent was supplied by the SPA Company in Milan.

We used the fowl pox strain of virus, of the gallinaceous type, adapted to eggs. It was of low virulence and we kept it in our laboratory. The degree of infection of this strain (15% suspension buffered in a homogenated physiological solution of infected membranes), determined on the chorion allantoideum of chicken embryos was $10^{5.5}$ (median infective dose of 0.1 milliliters).

Research on egg embryos. We performed two experiments: in the first, lysozyme was placed in direct contact with the virus and then injected; in the second experiment, the virus and the lysozyme were inoculated or injected separately.

In the first experiment, different concentrations of lysozyme chloride (5, 10, and 20 milligrams in 0.1 milliliter) were placed in contact, in equal volume, with 100 median infective dose of 0.1 milliliter of the virus, and after allowing this to remain for 8 and 24 hours at ambient temperature (22 degrees centigrade), 10 eggs, at their chorion allantoideum membrane, were inoculated with each type of mixture.

At the same time and under the same experimental conditions, a group of control eggs were inoculated in this way: first with lysozyme in the concentration mentioned; then with the virus alone at 0.1 milliliter of total infective dose; and then with the solvent plus the virus. Results were interpreted 72 to 96 hours after inoculation.

In the second experiment, lysozyme at various concentrations of 5, 10 and 20 milligrams per 0.1 milliliter was inoculated or injected into a group of 10 eggs at the chorion allantoideum membrane before the virus (100 ID_{50}/0.1 ml), for 8 and 24 hours. A group of controls was also used in this experiment.

For visualization of the foci, the chorion allantoideum membranes were washed with a solution of 4 grams of mercury bichloride, 30 milliliters of absolute alcohol, and 60 milliliters of distilled water.

Research on chickens. The chickens used in these tests were divided into three groups of ten chickens each (group A, group B, and group C).

Each chicken in group A and in group B was inoculated in the right posterior limb muscle with lysozyme chloride in amounts of 10 and 25 milligrams in 0.4 milliliter solution every 12 hours, beginning immediately after the infection and continuing for another six consecutive days.

The chickens in group C served as the control group; they were inoculated with 0.4 ml of the solvent alone every 12 hours.

The infection was made by cutaneous scarification, by depositing 0.1 milliliter of the viral suspension in the same area where groups A and B were inoculated with lysozyme, and where control group C was inoculated with the solvent. The evolution of the exanthematic lesions was checked every 12 hours for a period of 15 days.

Results and Conclusions

With regard to the research conducted with egg embryos, lysozyme exercised no inhibitory action on the development of the virus in the chorion allantoideum membranes. As a matter of fact, no matter what the concentration of lysozyme was, the lesions in membranes inoculated with the mixture of virus and lysozyme, after 8 and 24 hours at ambient temperature were practically superimposable on those of the eggs of the control group. Furthermore, in the case of eggs in which lysozyme was injected 8 and 25 hours before the virus, no significant difference was found with respect to the control group. In these experiments, Fleming's enzyme showed no toxicity, even at higher concentrations.

As to the research conducted on chickens, it is noted that the infection of the fowl pox virus was seen in the exanthematic form only and that the cutaneous proliferations were localized to the scarred area, also in the control group of chickens. Results of these tests are shown graphically in the attached table. From an examination of these data, it may be concluded that lysozyme, in doses of 50 milligrams a day, influences cutaneous eruptions of fowl pox in chickens infected experimentally (Group B). In fact, except for slower development, in almost all cases treated, a reduced degree of proliferation and diffusion were seen in comparison to the control group and healing was more rapid. No difference was noted between the chickens treated with 20 milligrams of lysozyme daily (group A) and the control group of chickens (group C).

Therefore, the results obtained, although in agreement with Orfei's results (reference 1) and with Compagnucci's (reference 5), with regard to the egg embryos, are not in accord with the latter's findings on the matter of experiments on chickens.

Based on our findings, it may be concluded therefore that lysozyme administered parenterally to chickens under laboratory conditions, at doses of 50 milligrams a day to each for seven days, may favorably influence the development of fowl pox exanthematic manifestations.

Conversely, there is no inhibitory action to the development of the virus itself in the chorion allantoideum membranes. In order to establish however whether Fleming's enzyme is able to influence favorably the development of natural infections, it will be necessary to conduct experiments of a practical nature.

FOWL POX SKIN ERUPTIONS IN CHICKENS

Days	Group A										Group B										Group C									
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
III	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
IV	o	o	-	o	o	-	-	-	o	-	-	-	-	o	-	-	o	-	-	o	o	o	o	-	o	o	o	-	-	-
V	o	o	o	o	o	o	-	o	o	o	o	o	-	o	o	-	o	-	-	o	o	o	o	o	o	o	o	-	o	o
VI	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	-	o	o
Vb	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	-	o	o
VIII	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	-	o	o
IX	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	-	o	o
X	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	-	o	o

o Initial eruptions
◎ Proliferative eruptions
● Confluent proliferative eruptions

N. B. In successive control groups, at the end of 15 days, a progressive attenuation or lessening of exanthematic lesions was observed, more evident in chickens of group B.

BIBLIOGRAPHY

1. Orfei, Z., Rend. Ist Sup. Sanità, 18, 426 (1955).

2. Mussa, B., Atti I Symp. Int. sul Lisozima, 261 (1959).

3. Naranjo, P., and De la Torre, F., Atti II Symp. Int. sul Lisozima, 23/II (1961).

4. Di Carlo, E. A., Ibidem, 57/II.

5. Compagnucci, M., Alosi, C., and Alby, B., Ibidem, 63/II.